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(FILE 'HOME' ENTERED AT 14:16:40 ON 13 DEC 2001)

FILE 'MEDLINE, AGRICOLA, CANCERLIT, SCISEARCH, CAPLUS, BIOSIS, MEDICONF'
ENTERED AT 14:16:49 ON 13 DEC 2001

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L1         17550 S ECDYSONE OR ECDYSTEROID
L2          2397 S L1 AND RECEPTOR
L3          2398 S L1 AND RECEPTOR?
L4            98 S L3 AND GLUCOCORTICOID
L5            71 DUP REM L4 (27 DUPLICATES REMOVED)
L6            31 S L5 AND PY<=1996
L7            31 SORT L6 PY
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FILE 'STNGUIDE' ENTERED AT 14:32:22 ON 13 DEC 2001

FILE 'MEDLINE, AGRICOLA, CANCERLIT, SCISEARCH, CAPLUS, BIOSIS, MEDICONF'
ENTERED AT 14:34:00 ON 13 DEC 2001

FILE 'STNGUIDE' ENTERED AT 14:34:58 ON 13 DEC 2001

FILE 'MEDLINE, AGRICOLA, CANCERLIT, SCISEARCH, CAPLUS, BIOSIS, MEDICONF'
ENTERED AT 14:38:15 ON 13 DEC 2001

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L8           330 S L3 AND (RESPONSE ELEMENT?)
L9            29 S L8 AND GLUCOCORTICOID?
L10           21 DUP REM L9 (8 DUPLICATES REMOVED)
L11           21 SORT L10 PY
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=> d an ti so au ab pi 12 17

L11 ANSWER 12 OF 21 CAPLUS COPYRIGHT 2001 ACS
AN 1997:684510 CAPLUS
DN 128:960
TI Hormone-mediated methods for modulating expression of exogenous genes in mammalian systems using **ecdysone receptor** fusion proteins
SO PCT Int. Appl., 105 pp.
CODEN: PIXXD2
IN Evans, Ronald M.; No, David
AB Mammalian expression systems using a modified **ecdysone receptor** to regulate expression of the foreign gene from an **ecdysteroid**-responsive promoter are described. Modified homo- and heterodimeric **ecdysone receptors**, modified ecdysterone **response elements**, transfer vectors and transgenic animals are described. Fusion proteins of **ecdysone receptors** and other hormone **receptors** contg. the **ecdysone receptor** ligand-binding domain, a DNA-binding domain, and the transcription activating domain of a mammalian hormone **receptor**, e.g. RXR are described. The **ecdysone receptor** may form a heterodimer with a **receptor** such as RXR by incorporating the peptides needed for their specific interaction. In addn., the DNA binding domain of the **ecdysone receptor** may be modified to that of another steroid hormone **receptor**. The system is an alternative to the prior art tetracycline regulation system that uses a eukaryotic regulation mechanism and a naturally lipophilic compd. that is easier to administer than tetracycline. The system can also be optimized to avoid complications such as adventitious induction of gene expression through the farnesoid X **receptor**. Construction of such a system in animal cell lines is described. Induction ratios of .gtoreq.100-fold were achieved with muristerone at concns. as low as 100 nM for a .beta.-galactosidase reporter gene. Transgenic mouse lines in which T cell-specific induction of a reporter gene by **ecdysteroids** was possible were constructed.
PATENT NO. KIND DATE APPLICATION NO. DATE

Print selected from Online session

PI	WO 9738117	A1	19971016	WO 1997-US5330	19970327
	W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
	RW: GH, KE, LS, MW, SD, SZ, UG, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
	CA 2251466	AA	19971016	CA 1997-2251466	19970327
	AU 9725572	A1	19971029	AU 1997-25572	19970327
	CN 1215432	A	19990428	CN 1997-193597	19970327
	EP 910652	A1	19990428	EP 1997-917146	19970327
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				
	JP 2000508895	T2	20000718	JP 1997-536281	19970327

L11 ANSWER 17 OF 21 CAPLUS COPYRIGHT 2001 ACS
AN 1999:736508 CAPLUS
DN 131:356081
TI Formulations useful for modulating expression of exogenous genes in mammalian systems, and products related thereto
SO PCT Int. Appl., 90 pp.
CODEN: PIXXD2
IN Evans, Ronald M.; Saez, Enrique
AB In accordance with the present invention, there are provided various methods for modulating the expression of an exogenous gene in a mammalian subject employing modified **ecdysone receptors**. Also provided are modified **ecdysone receptors**, as well as homomeric and heterodimeric **receptors** contg. same, nucleic acids encoding invention modified **ecdysone receptors**, modified hormone **response elements**, gene transfer vectors, recombinant cells, and transgenic animals contg. nucleic acids encoding invention modified **ecdysone receptor**.

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 9958155	A1	19991118	WO 1999-US8381	19990416
	W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
	RW: GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
	AU 9936486	A1	19991129	AU 1999-36486	19990416
	EP 1076569	A1	20010221	EP 1999-918614	19990416
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				

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L5 71 DUP REM L4 (27 DUPLICATES REMOVED)
L6 31 S L5 AND PY<=1996
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L10 21 DUP REM L9 (8 DUPLICATES REMOVED)
L11 21 SORT L10 PY

=> d an ti so au ab pi 1 3 5 9 12 17 18 19 21

L11 ANSWER 1 OF 21 SCISEARCH COPYRIGHT 2001 ISI (R)
AN 91:557746 SCISEARCH
TI THE DROSOPHILA ECR GENE ENCODES AN **ECDYSONE RECEPTOR**,
A NEW MEMBER OF THE STEROID-**RECEPTOR** SUPERFAMILY
SO CELL, (1991) Vol. 67, No. 1, pp. 59-77.
AU KOELLE M R (Reprint); TALBOT W S; SEGRAVES W A; BENDER M T; CHERBAS P;
HOGNESS D S
AB The steroid hormone **ecdysone** triggers coordinate changes in Drosophila tissue development that result in metamorphosis. To advance our understanding of the genetic regulatory hierarchies controlling this tissue response, we have isolated and characterized a gene, EcR, for a new steroid **receptor** homolog and have shown that it encodes an **ecdysone receptor**. First, EcR protein binds active **ecdysteroids** and is antigenically indistinguishable from the **ecdysone**-binding protein previously observed in extracts of Drosophila cell lines and tissues. Second, EcR protein binds DNA with high specificity at **ecdysone response elements** . Third, **ecdysone**-responsive cultured cells express EcR, whereas **ecdysone**-resistant cells derived from them are deficient in EcR. Expression of EcR in such resistant cells by transfection restores their ability to respond to the hormone. As expected, EcR is nuclear and found in all **ecdysone** target tissues examined. Furthermore, the EcR gene is expressed at each developmental stage marked by a pulse of **ecdysone**.

L11 ANSWER 3 OF 21 SCISEARCH COPYRIGHT 2001 ISI (R)
AN 92:430711 SCISEARCH
TI **ECDYSTEROID**-DEPENDENT REGULATION OF GENES IN MAMMALIAN-CELLS BY
A DROSOPHILA **ECDYSONE RECEPTOR** AND CHIMERIC
TRANSACTIVATORS
SO PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF
AMERICA, (15 JUL 1992) Vol. 89, No. 14, pp. 6314-6318.
ISSN: 0027-8424.
AU CHRISTOPHERSON K S; MARK M R; BAJAJ V; GODOWSKI P J (Reprint)
AB Steroid **receptors** are members of a large family of transcription factors whose activity is tightly regulated by the binding

of their cognate steroid ligand. Mammalian steroid hormone **receptors** have been exploited to obtain the regulated expression of heterologous genes in mammalian cells. However, the utility of these systems in cultured cells and transgenic animals is limited by the presence of endogenous steroids and their **receptors**. We show that a Drosophila **ecdysone receptor** can function in cultured mammalian cells as an **ecdysteroid**-dependent transcription factor. The activity of the **ecdysone receptor** was not induced by any of the mammalian steroid hormones tested. The DNA-binding and transactivation activities of viral, mammalian, or bacterial proteins were rendered **ecdysteroid** -dependent when fused to the ligand-binding domain of the **ecdysone receptor**. The **ecdysone receptor** may prove useful in selectively regulating the expression of endogenous or heterologous genes in mammalian cells.

L11 ANSWER 5 OF 21 SCISEARCH COPYRIGHT 2001 ISI (R)
AN 93:289079 SCISEARCH
TI STRUCTURAL FEATURES CRITICAL TO THE ACTIVITY OF AN **ECDYSONE RECEPTOR**-BINDING SITE
SO INSECT BIOCHEMISTRY AND MOLECULAR BIOLOGY, (JAN 1993) Vol. 23, No. 1, pp. 105-114.
ISSN: 0965-1748.
AU ANTONIEWSKI C (Reprint); LAVAL M; LEPESANT J A
AB Two **ecdysone-response elements** from the hsp27 (hsp27 EcRE) and the Fbp 1 (D EcRE) genes of Drosophila melanogaster were used as probes in a gel shift assay to investigate the interactions of the **ecdysone receptor** (EcR) with its cognate DNA **response element**. The source of EcR was a nuclear extract from the late third-larval instar fat body. The hsp27 and D EcREs share a sequence similarity at 12 positions over a 15bp region including an imperfect palindromic structure consisting of two pentamer half-sites separated by a single intervening nucleotide. We have shown that a short oligonucleotide containing this 11bp imperfect palindrome of the hsp27 EcRE and three flanking bp on each side is an efficient EcR binding site. Mutational analysis confirms that the integrity of both these half-sites as well as their 1bp spacing are critical for binding of the **ecdysone receptor**. The D EcRE behaved as a much weaker EcR binding site than the hsp27 EcRE but a single bp substitution was sufficient to confer upon it a binding capacity equivalent to that of the hsp27 EcRE. These results have led us to propose the sequence PuG(G/T)T(C/G)A(N)TG(C/A)(C/A)(C/t)Py as a revised version of a previously proposed EcRE consensus sequence.

L11 ANSWER 9 OF 21 SCISEARCH COPYRIGHT 2001 ISI (R)
AN 94:407716 SCISEARCH
TI THE **ECDYSONE** RESPONSE ENHANCER OF THE FBP1 GENE OF DROSOPHILA-MELANOGASTER IS A DIRECT TARGET FOR THE ECR/USP NUCLEAR **RECEPTOR**
SO MOLECULAR AND CELLULAR BIOLOGY, (JUL 1994) Vol. 14, No. 7, pp. 4465-4474.
ISSN: 0270-7306.
AU ANTONIEWSKI C; LAVAL M; DAHAN A; LEPESANT J A (Reprint)
AB The transcription of the Drosophila melanogaster Fbp1 gene is induced by the steroid hormone 20-hydroxyecdysone and restricted to the late-third-instar fat body tissue. In a previous study we showed that the -68 to -138 region relative to the transcription start site acts as an **ecdysone**-dependent third-instar fat body-specific enhancer in a transgenic assay. Here we report that seven nucleoprotein complexes are formed in vitro on this enhancer when a nuclear extract from late-third-instar fat body is used in a gel shift assay. Accurate mapping of the binding sites of the complexes revealed a remarkably symmetrical organization. Using specific antibodies, one of the complexes was identified as a heterodimer consisting of the **ecdysone receptor** (EcR) and Ultraspiracle (USP) proteins. The binding site

of the heterodimer as defined by mutagenesis and methylation interference experiments bears strong sequence similarity to the canonical hsp27 **ecdysone response element**, including an imperfect palindromic structure. The two elements diverge at three positions in both half-sites, indicating that the structure of an active EcR/USP binding site allows considerable sequence variations. In vivo footprinting experiments using ligation-mediated PCR and wild-type or **ecdysteroid**-deficient larvae show that occupancy of the Fbp1 EcR/USP binding site and adjacent region is dependent on a high concentration of **ecdysteroids**. These results provide strong evidence for a direct role of the EcR/USP heterodimer in driving gene expression in response to changes of the **ecdysteroid** titer during Drosophila larval development.

L11 ANSWER 12 OF 21 CAPLUS COPYRIGHT 2001 ACS
AN 1997:684510 CAPLUS
DN 128:960
TI Hormone-mediated methods for modulating expression of exogenous genes in mammalian systems using **ecdysone receptor** fusion proteins
SO PCT Int. Appl., 105 pp.
CODEN: PIXXD2
IN Evans, Ronald M.; No, David
AB Mammalian expression systems using a modified **ecdysone receptor** to regulate expression of the foreign gene from an **ecdysteroid**-responsive promoter are described. Modified homo- and heterodimeric **ecdysone receptors**, modified ecdysterone **response elements**, transfer vectors and transgenic animals are described. Fusion proteins of **ecdysone receptors** and other hormone **receptors** contg. the **ecdysone receptor** ligand-binding domain, a DNA-binding domain, and the transcription activating domain of a mammalian hormone **receptor**, e.g. RXR are described. The **ecdysone receptor** may form a heterodimer with a **receptor** such as RXR by incorporating the peptides needed for their specific interaction. In addn., the DNA binding domain of the **ecdysone receptor** may be modified to that of another steroid hormone **receptor**. The system is an alternative to the prior art tetracycline regulation system that uses a eukaryotic regulation mechanism and a naturally lipophilic compd. that is easier to administer than tetracycline. The system can also be optimized to avoid complications such as adventitious induction of gene expression through the farnesoid X **receptor**. Construction of such a system in animal cell lines is described. Induction ratios of .gtoreq.100-fold were achieved with muristerone at concns. as low as 100 nM for a .beta.-galactosidase reporter gene. Transgenic mouse lines in which T cell-specific induction of a reporter gene by **ecdysteroids** was possible were constructed.

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 9738117	A1	19971016	WO 1997-US5330	19970327
W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, KE, LS, MW, SD, SZ, UG, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
CA 2251466	AA	19971016	CA 1997-2251466	19970327
AU 9725572	A1	19971029	AU 1997-25572	19970327
CN 1215432	A	19990428	CN 1997-193597	19970327
EP 910652	A1	19990428	EP 1997-917146	19970327
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT,				

IE, FI
JP 2000508895 T2 20000718 JP 1997-536281 19970327

L11 ANSWER 17 OF 21 CAPLUS COPYRIGHT 2001 ACS
AN 1999:736508 CAPLUS
DN 131:356081
TI Formulations useful for modulating expression of exogenous genes in mammalian systems, and products related thereto
SO PCT Int. Appl., 90 pp.
CODEN: PIXXD2
IN Evans, Ronald M.; Saez, Enrique
AB In accordance with the present invention, there are provided various methods for modulating the expression of an exogenous gene in a mammalian subject employing modified **ecdysone receptors**. Also provided are modified **ecdysone receptors**, as well as homomeric and heterodimeric **receptors** contg. same, nucleic acids encoding invention modified **ecdysone receptors**, modified hormone **response elements**, gene transfer vectors, recombinant cells, and transgenic animals contg. nucleic acids encoding invention modified **ecdysone receptor**.

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 9958155	A1	19991118	WO 1999-US8381	19990416
W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
AU 9936486	A1	19991129	AU 1999-36486	19990416
EP 1076569	A1	20010221	EP 1999-918614	19990416
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				

L11 ANSWER 18 OF 21 CAPLUS COPYRIGHT 2001 ACS
AN 2000:821002 CAPLUS
DN 135:147922
TI Reporter-linked monitoring of transgene expression in living cells using the **ecdysone**-inducible promoter system
SO Eur. J. Cell Biol. (2000), 79(9), 653-657
CODEN: EJCBDN; ISSN: 0171-9335
AU Luers, Georg Hermann; Jess, Nicole; Franz, Thomas
AB Inducible promoter systems such as the **ecdysone**-inducible system or the tetracycline-regulated expression systems have proven to be powerful tools in studying gene function. In practice, such systems have met with the difficulty that either the vector expressing the transactivator gene or the vector carrying the **response element** are frequently silenced by flanking genomic sequences after stable integration. In order to identify those cells in a heterogeneous population in which a transgene is expressed from an **ecdysone**-inducible promoter, we have created the vector p2ER-EGFP/mcs that contains two **ecdysone**-inducible expression cassettes in tandem. Using two reporter genes, lacZ and green fluorescent protein (EGFP), we demonstrate that the expression of both genes can be co-induced from a very low baseline in CHO cells expressing the modified **ecdysone receptor** and the retinoid X **receptor**.
The expression of EGFP and lacZ from vector p2ER-EGFP/lacZ follows the same Muristerone A concn.-dependence as that of EGFP from vector pER-EGFP, indicating that the juxtaposition of the two inducible promoters in vector p2ER-EGFP/mcs does not cause cross interference between them. We suggest that this modification of the **ecdysone**-inducible promoter system

will allow for the visual control of the induced expression of other genes by Muristerone A.

L11 ANSWER 19 OF 21 CAPLUS COPYRIGHT 2001 ACS
AN 2000:161479 CAPLUS
DN 132:204016
TI Adenoviral vectors and inducible expression system for gene expression and therapy
SO PCT Int. Appl., 75 pp.
CODEN: PIXXD2
IN Mehtali, Majid; Sorg-guss, Tania
AB The invention concerns an inducible expression system using nucleotide sequences coding for a transcriptional activator of eukaryotic or viral origin and a recombinant adenoviral vector comprising a gene of interest placed under the control of a promoter inducible in trans by said transcriptional activator. The invention also concerns a recombinant adenoviral vector bearing a first expression cassette coding for a transcriptional activator and a second cassette bearing a gene of interest placed under the control of a promoter inducible in trans by said transcriptional activator. The invention further concerns an infectious viral particle, its prepn. method, a eukaryotic cell and a pharmaceutical compn. comprising such a vector or expression system as well as their use for therapeutic or prophylactic purposes. Thus, an adenoviral vector contg. genes for **glucocorticoid receptor** GRDEX and for blood-coagulation factor IX regulated by GRE sequences was prepd. Factor IX gene expression was induced in vitro and in vivo by dexamethasone.

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 2000012741	A2	20000309	WO 1999-FR2051	19990827
WO 2000012741	A3	20000504		
W: AU, CA, JP, US				
RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
FR 2782732	A1	20000303	FR 1998-10842	19980828
AU 9954262	A1	20000321	AU 1999-54262	19990827
EP 1108051	A2	20010620	EP 1999-940240	19990827
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				

L11 ANSWER 21 OF 21 CAPLUS COPYRIGHT 2001 ACS
AN 2001:618285 CAPLUS
DN 135:176717
TI A ligand dependent nuclear **receptors** transactivation system for screening insecticidal compds
SO PCT Int. Appl., 84 pp.
CODEN: PIXXD2
IN Tran, Hiep Tuan; Askari, Hossein; Schwartz, Michael; Butt, Tauseef
AB A yeast-based system is provided for identifying new mols. which activate nuclear **receptors** in a ligand-dependent fashion. A ligand dependent transactivation system for screening insecticidal compds. comprises: (a) a first DNA construct having a nucleic acid mol. encoding an altered **ecdysone receptor** operably linked to a promoter; (b) a second DNA construct having a nucleic acid mol. encoding a **receptor**, which heterodimerizes with said **ecdysone receptor** upon transactivation, said nucleic acid being operably linked to a promoter; (c) a third DNA construct comprising a promoter contg. a plurality of **ecdysone response elements**, said promoter being operably linked to a reporter gene; (d) a fourth DNA construct encoding a co-activator mol., said co-activator mol. being operably linked to a promoter sequence; and (e) a host cell comprising said first, second, third and fourth DNA constructs, expression of said reporter gene being dependent upon ligand dependent transactivation effectuated by said insecticidal compds. In a preferred embodiment, a method is provided utilizing **ecdysone**

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receptor, USP and GRIP I encoding expression vectors which may be used to advantage for screening new and useful insecticidal compds., detecting insecticidal residues as well as to regulate expression of a gene of interest in a host in a ligand-dependent manner.

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 2001061350	A1	20010823	WO 2001-US5429	20010220

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM

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L6 31 S L5 AND PY<=1996
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L7 ANSWER 4 OF 31 SCISEARCH COPYRIGHT 2001 ISI (R)
AN 91:557746 SCISEARCH
TI THE DROSOPHILA ECR GENE ENCODES AN **ECDYSONE RECEPTOR**, A NEW MEMBER OF THE STEROID-**RECEPTOR** SUPERFAMILY
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AU KOELLE M R (Reprint); TALBOT W S; SEGRAVES W A; BENDER M T; CHERBAS P; HOGNESS D S
AB The steroid hormone **ecdysone** triggers coordinate changes in Drosophila tissue development that result in metamorphosis. To advance our understanding of the genetic regulatory hierarchies controlling this tissue response, we have isolated and characterized a gene, EcR, for a new steroid **receptor** homolog and have shown that it encodes an **ecdysone receptor**. First, EcR protein binds active **ecdysteroids** and is antigenically indistinguishable from the **ecdysone**-binding protein previously observed in extracts of Drosophila cell lines and tissues. Second, EcR protein binds DNA with high specificity at **ecdysone** response elements. Third, **ecdysone**-responsive cultured cells express EcR, whereas **ecdysone**-resistant cells derived from them are deficient in EcR. Expression of EcR in such resistant cells by transfection restores their ability to respond to the hormone. As expected, EcR is nuclear and found in all **ecdysone** target tissues examined. Furthermore, the EcR gene is expressed at each developmental stage marked by a pulse of **ecdysone**.

L7 ANSWER 8 OF 31 SCISEARCH COPYRIGHT 2001 ISI (R)
AN 91:72614 SCISEARCH
TI IDENTIFICATION OF **ECDYSONE** RESPONSE ELEMENTS BY ANALYSIS OF THE DROSOPHILA EIP28/29 GENE
SO GENES & DEVELOPMENT, (**1991**) Vol. 5, No. 1, pp. 120-131.
AU CHERBAS L (Reprint); LEE K; CHERBAS P
AB We have identified **ecdysone**-response elements (EcREs) by studying regulation of the steroid-responsive Drosophila Eip28/29 gene. First, functional assays of deletion mutants identified large sequence regions required for the response; then a blotting method using the specifically labeled steroid **receptor** as probe identified **receptor**-binding regions. Three short **receptor**-binding regions near Eip28/29 have been identified: Prox and Dist]521 and 2295 nucleotides, respectively, downstream of the poly(A) site] are probably required for the Eip28/29 response in cell lines; Upstream (-440) is unnecessary for that response. We have also demonstrated that an EcRE-containing region from hsp27 contains a **receptor**-binding site. Each of these four **receptor**-binding regions functions as an EcRE when placed upstream of an **ecdysone** nonresponsive promoter and each contains an imperfect palindrome, suggesting the consensus 5'-RG(GT)TCANTGA(CA)CY-3'. Furthermore, a synthetic 15-bp fragment containing an imperfect palindrome similar to the consensus is a fully functional EcRE. The presence of any of the EcREs leads, in the absence of hormone, to depressed gene expression. When hormone is added,

it relieves this repression and causes additional activation. The similarity of the EcRE sequence is response elements for estrogen, thyroid hormone, and retinoic acid **receptors** suggests that the steroid **receptors** and their signal transduction mechanisms have been strongly and broadly conserved.

L7 ANSWER 9 OF 31 SCISEARCH COPYRIGHT 2001 ISI (R)
AN 92:430711 SCISEARCH
TI **ECDYSTEROID**-DEPENDENT REGULATION OF GENES IN MAMMALIAN-CELLS BY A DROSOPHILA **ECDYSONE RECEPTOR** AND CHIMERIC TRANSACTIVATORS
SO PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (**15 JUL 1992**) Vol. 89, No. 14, pp. 6314-6318.
ISSN: 0027-8424.
AU CHRISTOPHERSON K S; MARK M R; BAJAJ V; GODOWSKI P J (Reprint)
AB Steroid **receptors** are members of a large family of transcription factors whose activity is tightly regulated by the binding of their cognate steroid ligand. Mammalian steroid hormone **receptors** have been exploited to obtain the regulated expression of heterologous genes in mammalian cells. However, the utility of these systems in cultured cells and transgenic animals is limited by the presence of endogenous steroids and their **receptors**. We show that a Drosophila **ecdysone receptor** can function in cultured mammalian cells as an **ecdysteroid**-dependent transcription factor. The activity of the **ecdysone receptor** was not induced by any of the mammalian steroid hormones tested. The DNA-binding and transactivation activities of viral, mammalian, or bacterial proteins were rendered **ecdysteroid** -dependent when fused to the ligand-binding domain of the **ecdysone receptor**. The **ecdysone receptor** may prove useful in selectively regulating the expression of endogenous or heterologous genes in mammalian cells.

L7 ANSWER 13 OF 31 MEDLINE
AN 96265166 MEDLINE
TI Mutational analysis of the interaction between **ecdysteroid receptor** and its response element.
SO JOURNAL OF STEROID BIOCHEMISTRY AND MOLECULAR BIOLOGY, **(1993 Aug)** 46 (2) 135-45.
Journal code: AX4; 9015483. ISSN: 0960-0760.
AU Ozyhar A; Pongs O
AB The interaction between the partially purified **ecdysteroid receptor** (EcR) and the mutated **ecdysteroid**-response element (EcRE) from the hsp27 gene promoter was studied using the gel retardation competition assay. The results suggest that the EcR-hsp27 EcRE contact sites are made predominantly by base pairs which are at positions -7, -6, -5, -2, -1 and +2, +5, +6 of the hsp27 EcRE palindrome. An increase or decrease in the spacing between the half-palindromes reduces the affinity of the hsp27 EcRE to the **receptor**, while a mutation of the central A/T base pair to C/G has practically no effect on EcR binding. Unlike the **glucocorticoid**-response element and the estrogen-response element, the base pairs placed at positions -3, -4 and +1, +3, +4 of the hsp27 EcRE palindrome can be mutated without effect on the EcR binding.

L7 ANSWER 15 OF 31 SCISEARCH COPYRIGHT 2001 ISI (R)
AN 93:289079 SCISEARCH
TI STRUCTURAL FEATURES CRITICAL TO THE ACTIVITY OF AN **ECDYSONE RECEPTOR**-BINDING SITE
SO INSECT BIOCHEMISTRY AND MOLECULAR BIOLOGY, (**JAN 1993**) Vol. 23, No. 1, pp. 105-114.
ISSN: 0965-1748.
AU ANTONIEWSKI C (Reprint); LAVAL M; LEPESANT J A
AB Two **ecdysone**-response elements from the hsp27 (hsp27 EcRE)

and the Fbp 1 (D EcRE) genes of Drosophila melanogaster were used as probes in a gel shift assay to investigate the interactions of the **ecdysone receptor** (EcR) with its cognate DNA response element. The source of EcR was a nuclear extract from the late third-larval instar fat body. The hsp27 and D EcREs share a sequence similarity at 12 positions over a 15bp region including an imperfect palindromic structure consisting of two pentamer half-sites separated by a single intervening nucleotide. We have shown that a short oligonucleotide containing this 11bp imperfect palindrome of the hsp27 EcRE and three flanking bp on each side is an efficient EcR binding site. Mutational analysis confirms that the integrity of both these half-sites as well as their 1bp spacing are critical for binding of the **ecdysone receptor**. The D EcRE behaved as a much weaker EcR binding site than the hsp27 EcRE but a single bp substitution was sufficient to confer upon it a binding capacity equivalent to that of the hsp27 EcRE. These results have led us to propose the sequence PuG(G/T)T(C/G)A(N)TG(C/A)(C/A)(C/t)Py as a revised version of a previously proposed EcRE consensus sequence.

L7 ANSWER 18 OF 31 SCISEARCH COPYRIGHT 2001 ISI (R)
AN 95:93545 SCISEARCH
TI STEROID-HORMONE **RECEPTORS** - ACTIVATORS OF GENE-TRANSCRIPTION
SO JOURNAL OF PEDIATRIC ENDOCRINOLOGY, (**OCT/DEC 1994**) Vol. 7, No. 4, pp. 275-282.
ISSN: 0334-018X.
AU BRINKMANN A O (Reprint)
AB Over the past three decades, a great deal;of evidence has accumulated in favor of the hypothesis that steroid hormones act via regulation of gene expression. The action is mediated by specific nuclear **receptor** proteins, which belong to a superfamily of ligand-modulated transcription factors that regulate homeostasis, reproduction, development and differentiation. This family includes **receptors** for steroid hormones, thyroid hormones, hormonal forms of vitamin A and D, peroxisomal activators, and **ecdysone**.

Molecular cloning and structure/function analyses have revealed that all members of the steroid/thyroid hormone/retinoic acid **receptor** family have a similar functional domain structure: a variable N-terminal region, which is involved in modulation of gene expression; a short well-conserved DNA-binding domain, which is crucial for recognition of specific DNA sequences and for **receptor** dimerization; and a partially conserved C-terminal ligand-binding domain, which is important for hormone binding and also for **receptor** dimerization and transactivation.

In contrast to other members of the **receptor** superfamiiy steroid hormone **receptors** form transient complexes with several heat shock proteins. This interaction promotes proper folding and stability of the **receptor** molecule. Hormone binding induces a conformational change in the **receptor** molecule and simultaneously a dissociation of all heat shock proteins, which results in DNA-binding of the hormone-**receptor** complex.

The hormone-**receptor** complex can be considered as a ligand-activated transcription factor, which regulates gene expression by binding to hormone-response elements which are located usually in the 5'-flanking sequences of the target genes. Hormone response elements are 12-18 base pair DNA sequences that are partially palindromic and consist of two ''half sites'', which are separated by a variable spacer. The primary nucleotide sequence of the hormone response element as well as the orientation and the spacing between the two half-sites are crucial for the specificity of the response to various hormone-**receptor** complexes. The binding of nuclear hormone **receptors** to their hormone response elements occurs as dimers: one **receptor** molecule binds to each half site. **Receptors** enhance transcription by stabilizing general transcription factors either directly at the TATA-box or through interactions with proteins bound to upstream

promoter sequences or via interaction with transcription intermediary factors which can be considered as coupling proteins between the **receptor** and other protein components in the transcription initiation complex.

For all steroid **receptors**, overwhelming evidence is reported for hyperphosphorylation of the **receptor** after ligand binding. In some cases, this can result in increased transcriptional activity. Phosphorylation can increase the negative charge and acidity of a region of a protein, thereby modifying interactions with other proteins or with DNA. Hypo- and hyperphosphorylation at the same time in different regions of steroid **receptor** molecules might provide a mechanism for differential transcription regulation of certain genes, in addition to host cell and promoter context of the genes to be transcribed or repressed.

Disruption of nuclear **receptor** function is implicated in a number of hormone resistance syndromes. **Receptor** gene defects have frequently been shown to be the cause of several forms of androgen insensitivity, Vitamin D resistant forms of rickets and partial cortisol resistance.

L7 ANSWER 21 OF 31 SCISEARCH COPYRIGHT 2001 ISI (R)
AN 94:407716 SCISEARCH
TI THE **ECDYSONE** RESPONSE ENHANCER OF THE FBP1 GENE OF DROSOPHILA-MELANOGASTER IS A DIRECT TARGET FOR THE ECR/USP NUCLEAR **RECEPTOR**
SO MOLECULAR AND CELLULAR BIOLOGY, (**JUL 1994**) Vol. 14, No. 7, pp. 4465-4474.
ISSN: 0270-7306.
AU ANTONIEWSKI C; LAVAL M; DAHAN A; LEPESANT J A (Reprint)
AB The transcription of the Drosophila melanogaster Fbp1 gene is induced by the steroid hormone 20-hydroxyecdysone and restricted to the late-third-instar fat body tissue. In a previous study we showed that the -68 to -138 region relative to the transcription start site acts as an **ecdysone**-dependent third-instar fat body-specific enhancer in a transgenic assay. Here we report that seven nucleoprotein complexes are formed in vitro on this enhancer when a nuclear extract from late-third-instar fat body is used in a gel shift assay. Accurate mapping of the binding sites of the complexes revealed a remarkably symmetrical organization. Using specific antibodies, one of the complexes was identified as a heterodimer consisting of the **ecdysone** **receptor** (EcR) and Ultraspiracle (USP) proteins. The binding site of the heterodimer as defined by mutagenesis and methylation interference experiments bears strong sequence similarity to the canonical hsp27 **ecdysone** response element, including an imperfect palindromic structure. The two elements diverge at three positions in both half-sites, indicating that the structure of an active EcR/USP binding site allows considerable sequence variations. In vivo footprinting experiments using ligation-mediated PCR and wild-type or **ecdysteroid**-deficient larvae show that occupancy of the Fbp1 EcR/USP binding site and adjacent region is dependent on a high concentration of **ecdysteroids**. These results provide strong evidence for a direct role of the EcR/USP heterodimer in driving gene expression in response to changes of the **ecdysteroid** titer during Drosophila larval development.

L7 ANSWER 22 OF 31 BIOSIS COPYRIGHT 2001 BIOSIS
AN 1994:495783 BIOSIS
TI Phylogeny of the steroid **receptor** superfamily.
SO Molecular Phylogenetics and Evolution, (1994) Vol. 3, No. 3, pp. 192-205.
ISSN: 1055-7903.
AU Detera-Wadleigh, Sevilla D. (1); Fanning, Thomas G.
AB The phylogenetic relationships of 56 nuclear hormone **receptors** from both invertebrates and vertebrates were determined by the parsimony method (PAUP). The consensus tree suggests that the ancestral gene diverged into five major subfamilies, each of which evolved into at least

one cluster of related molecules. These subfamilies are represented by: (i) thyroid hormone **receptors** (TR); (ii) steroid **receptors** (SR); (iii) retinoic acid **receptors** (RAR), retinoid X **receptors** (RXR), and the chicken ovalbumin upstream promoter transcription factor 1 (COUP) group; (ix) peroxisome proliferator-activated **receptors** (PPAR); and (v) vitamin D **receptor** (VDR) and knirps (kni) group. Although the neighbor-joining (N-J) method clustered the **receptors** into a greater number of subfamilies, it was evident that the components of the terminal **receptor** subgroups were similar to those found in the PAUP tree. These terminal clusters might then represent phylogenetically stable relationships. The positions of some orphan **receptors** were perturbed when a different algorithm was employed in the analysis. Both PAUP and N-J evolutionary trees showed that the **receptors** within the subgroups of a major sublineage tend to recognize hormones of very similar structure. This finding suggests that the relative phylogenetic position of orphans to well-characterized **receptors** might be exploited to predict the type of ligand they would recognize.

L7 ANSWER 26 OF 31 SCISEARCH COPYRIGHT 2001 ISI (R)
AN 95:374476 SCISEARCH
TI CRYSTAL-STRUCTURE OF THE LIGAND-BINDING DOMAIN OF THE HUMAN NUCLEAR **RECEPTOR** RXR-ALPHA
SO NATURE, (**01 JUN 1995**) Vol. 375, No. 6530, pp. 377-382.
ISSN: 0028-0836.
AU BOURGUET W; RUFF M; CHAMBON P; GRONEMEYER H; MORAS D (Reprint)
AB The crystal structure of the human retinoid-X **receptor** RXR-alpha ligand-binding domain reveals a previously undiscovered fold of an antiparallel alpha-helical sandwich, packed as dimeric units. Two helices and one loop form the homodimerization surface, and hydrophobic heptad repeats participate in stabilizing the fold. The existence of a ligand-binding pocket is proposed that would allow 9-cis retinoic acid to interact with different functional modules, including the AF-2 activating domain. Several lines of evidence indicate that the overall structure is a prototype fold of ligand-binding domains of nuclear **receptors**.

L7 ANSWER 28 OF 31 SCISEARCH COPYRIGHT 2001 ISI (R)
AN 95:229763 SCISEARCH
TI MOSQUITO **ECDYSTEROID RECEPTOR** - ANALYSIS OF THE CDNA AND EXPRESSION DURING VITELLOGENESIS
SO INSECT BIOCHEMISTRY AND MOLECULAR BIOLOGY, (**JAN 1995**) Vol. 25, No. 1, pp. 19-27.
ISSN: 0965-1748.
AU CHO W L; KAPITSKAYA M Z; RAIKHEL A S (Reprint)
AB An insect steroid hormone, 20-hydroxyecdysone (20E), plays an important role in regulating egg maturation in mosquitoes. To better understand its role, we cloned the cDNA coding for the putative **ecdysteroid receptor** from the mosquito, Aedes aegypti (AaEcR). The 4158 bp AaEcR cDNA has an open reading frame of 675 amino acids with 10 potential glycosylation sites and a putative phosphorylation polyserine domain. The AaEcR has a DNA binding domain with two zinc fingers and a ligand binding domain characteristic of members of the steroid hormone **receptor** superfamily. These AaEcR domains share 97 and 87% identities with the respective domains of the Drosophila **ecdysteroid receptor** (DmEcR). However, the A/B region of the AaEcR shares 35% identity with that of DmEcR-B1 isoform. The F region, located at the carboxyl-terminal of the AaEcR, has only 9% identity with the corresponding region of DmEcR. Potential nuclear targeting and dimerization signals are also present in the AaEcR sequence. There are three AaEcR transcripts of 4.2 kb, 6 kb and 11 kb in adult mosquitoes. 4.2 kb mRNA is predominantly expressed in female mosquitoes during vitellogenesis. In both the fat body and ovaries of the female mosquito, the level of AaEcR mRNA is high at the previtellogenic period and after the onset of vitellogenesis (6 h post blood meal, PBM).

L Number	Hits	Search Text	DB	Time stamp
1	677	ecdysone or ecdysteroid	USPAT; US-PGPUB; EPO; JPO; DERWENT	2001/12/13 14:58
7	442	(ecdysone or ecdysteroid) and (receptor$5 or $10element)	USPAT; US-PGPUB; EPO; JPO; DERWENT	2001/12/13 15:12
14	126	((ecdysone or ecdysteroid) and (receptor$5 or $10element)) and (DNA adj binding)	USPAT; US-PGPUB; EPO; JPO; DERWENT	2001/12/13 15:14
26	72	(((ecdysone or ecdysteroid) and (receptor$5 or $10element)) and (DNA adj binding)) and glucocorticoid$15	USPAT; US-PGPUB; EPO; JPO; DERWENT	2001/12/13 15:15

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